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The origin of two cryptic species of African desert jerboas (Dipodidae: *Jaculus*)

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The desert biota is exposed to extreme and variable conditions that shape its evolution and diversification processes. In this respect, the *Jaculus* jerboas have gained the attention of researchers as a result of their broad Saharan–Arabian distribution and their high and unexplained, morphological, anatomical, and molecular variation. In the present study, mitochondrial and nuclear genealogies where used to confirm monophyly of two cryptic species: *Jaculus jaculus* and *Jaculus deserti*. The reconstructed demography showed that the evolutionary histories of the species are markedly different and that the expansion into North-West Africa by *J. deserti* was more recent than that of *J. jaculus*. The weak ecological separation between species and the signs of recent population growth and expansion of *J. deserti* suggest that its sympatric occurrence with *J. jaculus* is recent and that these species evolved in isolated populations, after diverging around the Pliocene–Pleistocene boundary. The importance of climate changes on the Sahara Desert biota is discussed in the context of genetic diversification. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **105**, 435–445.

ADDITIONAL KEYWORDS: climate change – ecological niche – phylogeny – Sahara – speciation.

INTRODUCTION

Large geological structures, such as mountains and deserts, are often considered as barriers to migration, as is the case for the African Sahara Desert, which effectively prevents mixing of Eurasian and Afrotropical biotas. However, it is also a centre for the evolution of organisms that are among the most specialized in their adaptation to arid environments. The region hosts animals that can live in complete absence of access to fresh water. The obscure structure and dynamic history of desert environments, particularly the Sahara (Le Houérou, 1997; Gasse, 2000; Kröpelin *et al*., 2008), is assumed to have stimulated diversification of desert specialists and is probably responsible for their high intraspecific variability (Brito *et al*., 2009; Fonseca *et al*., 2009; Nicolas *et al*., 2009; Abiadh *et al*., 2010; Drake *et al*., 2010; Genner & Haesler, 2010; Perera & Harris, 2010). To understand the importance of desert microhabitat structuring on the evolution of these organisms, integrated ecological and genetic studies are needed. Such studies can provide information for interpreting and predicting population dynamics of the arid biota of these vulnerable regions in the face of ongoing climate change.

African jerboas (*Jaculus* spp., Erxleben, 1777, *Dipodidae*), comprising rodents well adapted to extreme conditions, inhabit different types of deserts and semi-arid environments from Senegal (*Jaculus jaculus*) in West Africa to Afghanistan and Pakistan (*Jaculus blanfordi*) (Holden & Musser, 2005; Shenbrot *et al*., 2008). The genus is represented by three species (*J. jaculus*, *Jaculus orientalis and J. blanfordi*), yet within-species variation is remarkable, which has lead to the recognition of several subspecies or species (e.g. within the lesser Egyptian jerboa) (Shahin, 2003; Holden & Musser, 2005; Shenbrot *et al*., 2008). The lesser Egyptian jerboa, *J. jaculus* (Linnaeus, 1758), populates most of North Africa and the Arabic peninsula up to south-western Iran (Amori *et al*., 2008; Aulagnier *et al*., 2009). The description of

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subspecies within *J. jaculus* was based primarily on morphological and anatomical differences between populations, referring to divergent dorsal coat colour morphs and other phenotypic traits (Gharaibeh, 1997; Shahin, 1999, 2003; Ben faleh *et al*., 2010). In most cases, morphological studies were not followed by ecological and genetic investigations, such that the possible adaptive signatures of variation and the evolutionary histories of lineages were not recognized (Shahin, 2003).

Among the best studied Saharan rodents are the jerboas of Tunisia, where two cryptic species are assumed to coexist in sympatry (Gharaibeh, 1997). The taxonomic separation of the two species, *J. jaculus* (Linnaeus, 1758) and *Jaculus deserti* Loche, 1867, was based on morphological studies (Ranck, 1968) but, recently, correspondence between morphological and genetic variation has been shown (Ben faleh *et al*., 2010). A division between the two lineages/cryptic species was found in mitochondrial (cytochrome *b*) sequences and in the frequencies of 23 loci encoding 16 enzymatic proteins (Ben faleh, Othmen & Said, 2010; Ben faleh *et al*., 2010). The study of enzymatic proteins, however, failed to describe species-diagnostic loci, which could be explained by hybridization and gene flow, homoplasy or incomplete lineage sorting. Therefore, it is unclear whether the differences between nuclear and mitochondrial markers are solely caused by a higher polymorphism in mitochondrial (mt)DNA or whether the nuclear genomes are homogenized (e.g. by interspecific gene flow).

The main aim of the present study was to reconstruct the evolutionary scenarios that underlie the observed patterns of divergence in mtDNA between African jerboas. We tested for signs of hybridization and gene flow between species in nuclear and mitochondrial markers because introgression is a relatively common phenomenon (e.g. of mtDNA) (Arnold, 2006; Melo-Ferreira *et al*., 2009; Runck, Matocq & Cook, 2009). We tested for the ecological signs of speciation processes because the sympatric species are expected to specialize on different ecological niches (Nosil, Harmon & Seehausen, 2009). Samples of African jerboas (including both putative cryptic species: *J. jaculus* and *J. deserti*), collected in North-West Africa, with localities ranging from north of the Senegal river, through the Atlantic coastal Sahara to the south of the Anti-Atlas mountains, were used to test these hypotheses. This area in Africa lacks major morphological features, such as high mountain ridges and wide rivers, which could prevent the migration of jerboas. We studied two unlinked genetic markers, one mitochondrial and one nuclear, and carried out ecological analyses aiming to: (1) identify the main evolutionary lineages and their divergence times; (2) describe the genetic structuring within species as well as their demographic histories; and (3) identify environmental preferences of the different lineages.

MATERIAL AND METHODS

Samples

Analyses were performed on 43 *Jaculus* individuals collected in Mauritania, Morocco, and Tunisia during field expeditions (Brito *et al*., 2010) (see Supporting information, Table S1). Samples likely included two cryptic species as previously described by Ben faleh *et al*. (2010). However, their recognition was problematic and the geographical distribution unknown. The geographical location of each sample was recorded with a Global Positioning System (GPS) on the WGS84 datum (Fig. 1).

Genetic analysis

Genomic DNA was extracted from ethanol preserved muscle or ear tissue using a Qiagen extraction protocol. Purification was conducted in a KingFisher apparatus. The genetic polymorphism of the full length (1140 bp) of the mitochondrial cytochrome *b* (*cyt b*) gene was analyzed. The *cyt b* gene was amplified using two primer pairs (each producing around 900 bp long overlapping sequences) designed for *Jaculus* species: Jac1F (5'-GGACTCCCCATGACC TAT-3'), Jac1R (5'-TGCTGGTTTACAAGACCA-3'), Jac4F (5'-CAAACCCACTTAATACGC-3'), and Jac4R (5'-CGAGAAGAGGGATACGAC-3'). Two sets of polymerase chain reaction (PCR) reactions were performed in 30-μL volume mixes containing 3 μL of DNA, 0.24 μL of DreamTaq polymerase (Fermentas; 0.025 U μL^{-1}), 1.8 μL of F and R primers (0.6 μM), 1.2 μL of $MgCl_2$ (2 and 2.5 mM; for Jac1 and Jac4 primers), 3 μL of dNTP (0.2 mM), 3 μL of reaction buffer, and 15.96 μL of H_2O. PCRs protocols included: 3 min of preliminary activation of Taq polymerase at 95 °C, followed by 35 three-step cycles of denaturation at 95 °C (30 s), annealing for 30 s at 58 °C and 51 °C for Jac 1 and Jac 4 respectively, extension at 72 °C (90 and 60 s; for Jac1 and Jac4), and a final extension at 72 °C (10 min). Sequence fragments of the von Willebrand factor (*vWF*) nuclear gene (874 bp) were amplified using primers: vWF-F (5'-CAAGGTTG ACCGGCCCGAA-3') and vWF-R (5'-GAGAAGC CGCGCTCCGAGAGGTAC-3'). PCR reactions were performed in 30-μL volume mixes containing 3 μL of DNA, 0.24 μL of DreamTaq polymerase (Fermentas; 0.025 U μL^{-1}), 1.8 μL of F and R primers (0.6 μM), 1.2 μL of $MgCl_2$ (2 mM), 3 μL of dNTP (0.2 mM), 3 μL of reaction buffer, and 15.96 μL of H_2O. PCR reactions were performed with protocols described for *cyt* b Jac1 primer with an annealing temperature of 48.6 °C. Sequencing reactions were performed using PCR

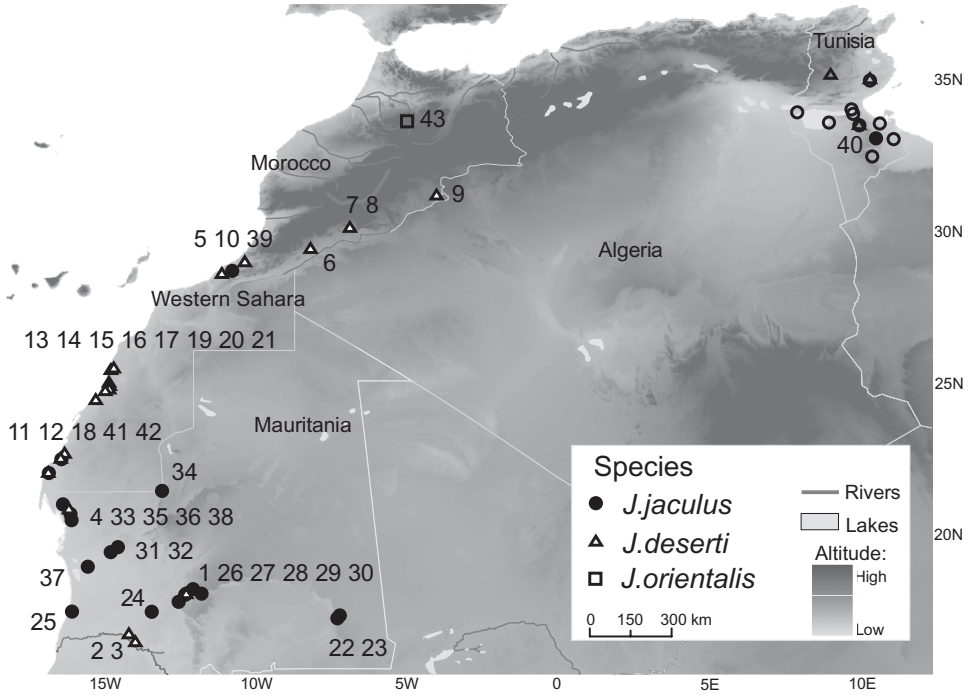

Figure 1. Geographical locations of samples included in the present study. Numbers refer to the sample codes included on phylogenetic trees. Uncoded samples locations originate from Ben faleh *et al*. (2010).

primers in 18-μL mixes containing 1 μL of primer (3.2 μM) and 3 μL of PCR purified product (Exo-SAP purification) in accordance with the ABI sequencing protocol (with 0.5 μL of 25× sequencing premix and 3.75 μL of 5× sequencing buffer). Sequencing was conducted in two directions using a BigDye Terminator kit (Applied Biosystems) on an Applied Biosystems 3130xl Genetic Analyzer. All sequences were merged using the SEQSCAPE, version 2.1.1 (Applied Biosystems SeqScape® Software http://www.appliedbiosystems.com) and aligned using CLUSTALX, version 2.0.10 (http://www.ebi.ac.uk/tools/clustalw2).

Phylogenetic analyses included sequences of three outgroup species, *J. orientalis*, *Dipus sagitta* and *Allactaga elater* (GenBank reference numbers for *cyt b*: JN214546, AM407909, AJ389534; for *vWF*: JN214562, AJ224665, AJ224661). Available sequences (in GenBank) of *cyt b* where included in a separate analysis. An Akaike information criterion based model selection was conducted for each dataset in MODELTEST, version 3.0 (Posada & Crandall, 1998) implemented in HYPHY (Kosakovsky *et al*., 2005). Neighbour-joining phylogenies were reconstructed and the robustness of the trees was assessed by bootstrap resampling in PHYLIP, version 3.68 (1000 replications; [Felsenstein, 1985]). The Maximum-likelihood phylogenetic analysis (Felsenstein, 1981) was performed using PHYML, version 2.4.4 with 1000 bootstrap resampling (Guindon & Gascuel, 2003). A Bayesian inference approach was applied to reconstruct the phylogeny using MrBayes, version 3.1.2 (Huelsenbeck & Ronquist, 2001). Four Markov chain Monte Carlo simulations were started from the tree with random topology and branch lengths. Simulations were run for ten million generations with trees sampled every 100 generations (100 000 trees saved) with the first 25 000 trees discarded from further analyses (burn-in). The remaining trees were used to construct the consensus tree and estimate posterior probabilities for all nodes in the Bayesian inference tree using the 50% majority rule. The estimations of the age of divergence between phylogenetic clades were based on median and mean substitution rates estimated for third codon position of the *cyt b* gene for mammals (Nabholz, Glémin & Galtier, 2008). The detailed relationships among *cyt b* haplotypes were analyzed and visualized by the statistical parsimony method implemented in TCS, version 1.21 (Clement, Posada & Crandall, 2000). The allelic phase of nuclear gene was determined with PHASE, version 2.1 (Stephens, Smith & Donnelly, 2001) and the haplotypes with phase probabilities at each site of 1.0 were used to describe sequence variation and between species differences.

The mismatch distributions for *cyt b* haplotypes were calculated (Rogers & Harpending, 1992) and goodness-of-fit tests of the observed versus expected

distributions according to the Sudden Expansion Model were tested in ARLEQUIN (Excoffier, Laval & Schneider, 2005). The Sudden Expansion Model assumes that initial population at equilibrium (of size: θ_0) expanded rapidly (new size: θ_1), mutational times ago: $\tau = 2ut$ (where u is the mutation rate and t is the time since the expansion in generations). The confidence intervals for τ were calculated with 1000 bootstrap replicates for $\alpha = 0.010$. One generation per year and mean and median substitution rates for mammals (Nabholz *et al*., 2008; no estimation of substitution rates is available for African jerboas) were assumed for estimation of the age since expansion. Assumptions of selective neutrality and population equilibrium were tested with Tajima's D and Fu's F_S statistics with 5000 simulations in ARLEQUIN (Excoffier *et al*., 2005).

Ecological analysis

Samples were characterized by environmental factors with the support of a geographical information system, ArcGIS (ESRI, 2008). These factors included annual precipitation and annual mean temperature, which were downloaded from Worldclim (http://www.worldclim.org/) at a spatial resolution of 0.0083 decimal degrees (Hijmans *et al*., 2005), and land cover from the years 2004–2006, downloaded from the European Space Agency Ionia GlobCover Portal (http://ionia1.esrin.esa.int/) at a spatial resolution of 0.0028 decimal degrees (Bicheron *et al*., 2008). Four land cover categories were present in the study area: closed to open (> 15%) herbaceous vegetation (grassland, savannas or lichens/mosses), bare areas, consolidated bare areas (rocky desert), and nonconsolidated bare areas (sandy desert). Geographical locations of samples were intersected with environmental grids using Hawth's analysis Tools (Beyer, 2006) implemented in the geographical information system. Differences in the climatic variants between localities where haplogroups were detected and differences in the number of occurrences of each haplogroup in each land-cover category were tested with t-tests and $r \times k$ χ^2 tests, respectively.

RESULTS

In total, 43 *Jaculus* samples were used in the analyses (Fig. 1; see also Supporting information,Table S1). For all samples, *cyt b* was sequenced and used in phylogenetic reconstructions. The *cyt b* sequences were most likely of mitochondrial origin and not of nuclear integrated copies because they align perfectly with the complete mtDNA sequence of *Jaculus* (GenBank accession number: NC_005314), no indels or codons stop were found in the dataset, the third position base composition was typical (A, 35.6%; C, 40.1%; G, 2.6%; T, 21.7%) of small mammals (A, 39%; C, 36%; G, 3%; T, 21%; (Johns & Avise, 1998) and the variation between codon positions was characteristic for coding regions (mean divergence over all sequence pairs for first, second, and third positions were: 0.009, 0.001, and 0.288, respectively). In total, 227 sites were polymorphic (80% positions were invariable), which defined 39 haplotypes (Table 1). The GTR model with a Gamma shape parameter (0.245) was chosen as the most appropriate model of substitution. The outcome of the phylogenetic analysis was congruent among all statistical methods (Fig. 2; for tree including GenBank sequences, see also Supporting information, Fig. S1). The samples split into monophyletic clades with very high support values (100, 100, and 1.0 for bootstrap values for maximum likelihood and Neighbour-joining trees and posterior probability for Bayesian method: Fig. 2). The TrM model with proportion of invariant sites (0.464) was the most appropriate model for *vWF* dataset. These analyses were conducted on sequences of *vWF* gene generated for 24 samples for which quality of extracted DNA allowed unambiguous and sufficient amplification. The sequences of the *vWF* nuclear gene representing

Table 1. Sequence diversity and neutrality tests for *cyt b* (1140 bp) and *vWF* (874 bp) genes

	Gene	N	n_h	H	n_p	π (%)	Tajima's D	$p_{\text{Tajima's D}}$	Fu's F_S	$p_{\text{Fu's Fs}}$
Jaculus jaculus and *Jaculus deserti*	*cyt b*	42	39	0.99	167	5.78(2.83)	2.04	0.988	–5.48	0.045
Jaculus deserti		21	18	0.98	34	0.42(0.24)	–1.93	0.016	–12.14	< 0.00001
Jaculus jaculus		21	21	1.0	62	1.10(0.58)	–1.12	0.124	–11.59	0.0002
Jaculus jaculus and *Jaculus deserti*	*vWF*	24	12	0.95	25	0.88(0.59)	0.24	0.656	–1.59	0.230
Jaculus deserti		12	7	0.91	14	0.55(0.33)	0.13	0.585	–0.14	0.448
Jaculus jaculus		12	5	0.94	7	0.40(0.27)	0.76	0.753	–1.01	0.173

N, number of samples; n_h, number of haplotypes; H, haplotype diversity; n_p, number of polymorphic sites; π, nucleotide diversity (and its SDs).

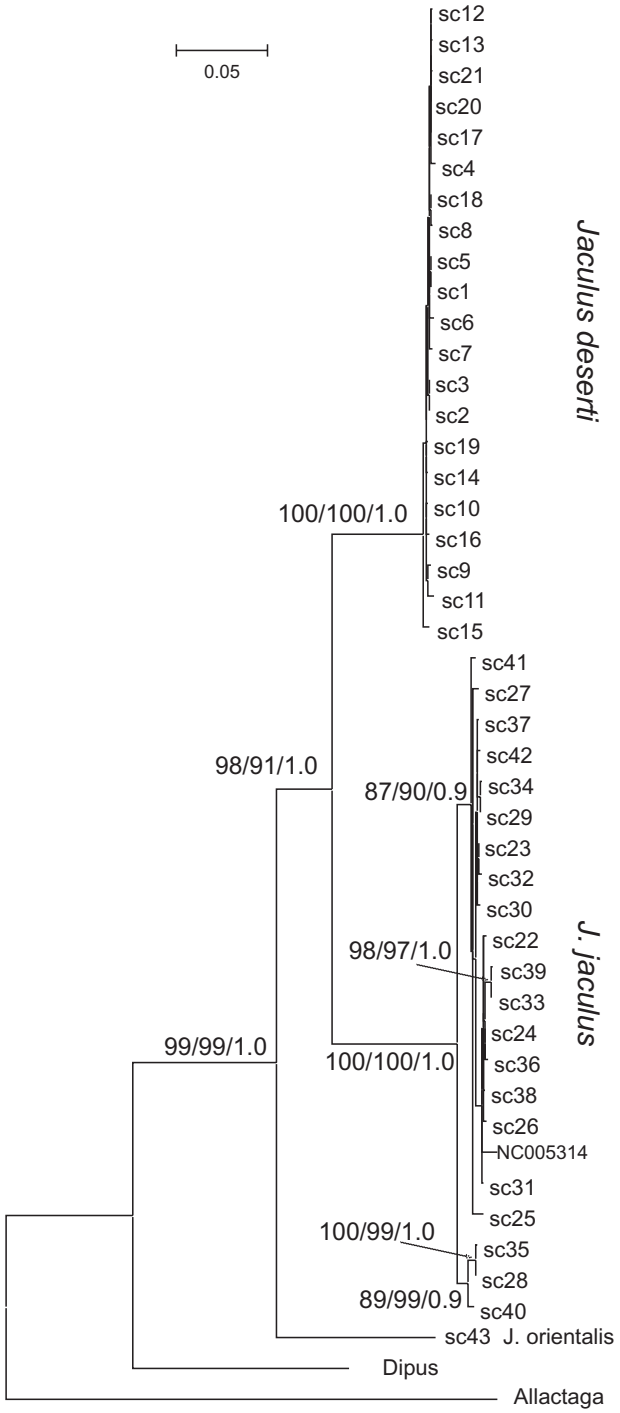

Figure 2. Consensus phylogram reconstructed from the cytochrome *b* (*cyt b*) sequences (1140 bp) using the maximum likelihood (ML) method. Numbers in the external tree nodes refer to codes of collected samples (Fig. 1; see also Supporting information, Table S1). Numbers in the internal nodes represent the percentage of bootstrap support for ML and Neighbour-joining analyses and Bayesian posterior probabilities. Branch length is proportional to the number of substitutions per site.

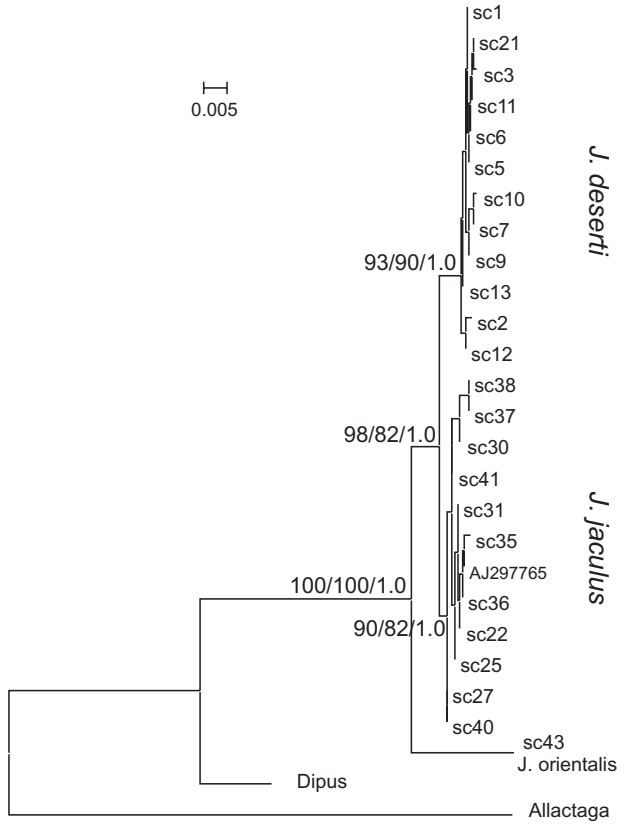

Figure 3. Consensus phylogram reconstructed from the von Willebrand factor (*vWF*) sequences (874 bp) using the maximum likelihood (ML) method. Numbers in the external tree nodes refer to sample codes (Fig. 1; see also Supporting information, Table S1). Numbers in the internal nodes represent the percentage of bootstrap support for ML and Neighbour-joining analyses and Bayesian posterior probabilities. Branch length is proportional to the number of substitutions per site.

both main mitochondrial haplogroups formed monophyletic clades (Fig. 3) similar to those reconstructed from mitochondrial genealogy. Analyses of concatenated sequences showed similar results (see also Supporting information, Fig. S2).

The sequence divergence value of *cyt b* sequences of putative cryptic species *J. jaculus* was higher than the diversity detected within *J. deserti* (Table 1). The *cyt b* sequences of *J. deserti* and *J. jaculus* differed by 13.2% (SE: 1.0) and 12.1% (SE: 1.0) of base pairs, respectively, from *J. orientalis* sequences, whereas the difference between *J. deserti* and *J. jaculus* was 10.6% (SE: 0.9, between group mean distances computed with the Kimura two-parameter method and its standard errors calculated by bootstrap method with 1000 replicates). The level of divergence between *J. deserti* and *J. jaculus* cryptic species referred to a time of divergence between 1.8 and 4.1 Mya.

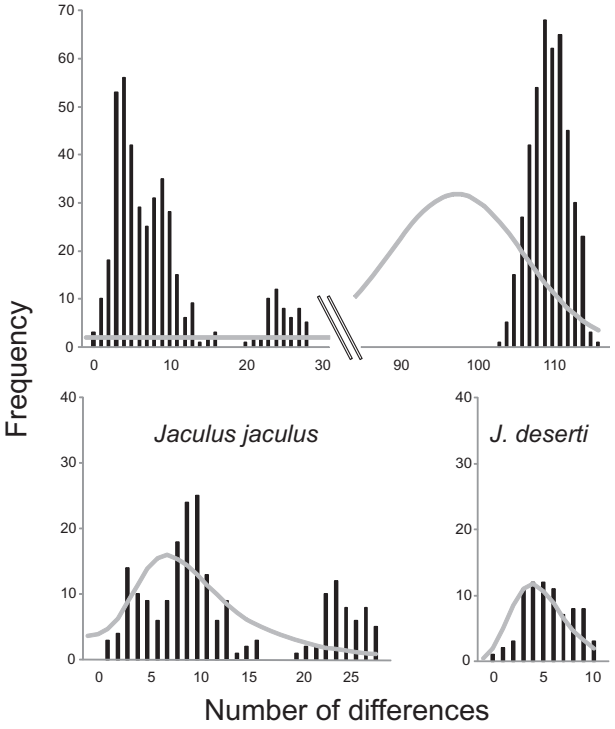

Figure 4. Observed (black bars) and expected (grey lines) mismatch distributions for cytochrome *b* (*cyt b*) haplotypes, including *Jaculus jaculus* and *Jaculus deserti* in combined (upper panel) and separate (lower panels) analyses.

Sequence diversity of the *vWF* gene was lower than that of the *cyt b* gene (Table 1), which was even more apparent at the interspecific level. Between species mean differences in *cyt b* were five-fold higher than those observed for *vWF* gene. *Jaculus deserti* and *J. jaculus vWF* sequences differed by 3.2% (SE: 0.6) and 3.0% (SE: 0.6) of base pairs, respectively, from *J. orientalis* sequences, whereas the difference between *J. deserti* and *J. jaculus* was 1.3% (SE: 0.3).

The mismatch distribution of the *cyt b* haplotypes showed a bimodality of the number of pairwise differences (Fig. 4), illustrating the existence of two divergent haplogroups. The mismatch analyzed separately for each of the mtDNA clades showed unimodality in distribution for *J. deserti* [τ (CI) = 4.38 (2.71–5.82); Θ_0 (CI) $\approx$ 0 (0–1.28); Θ_1 (CI) $\approx$ 99999 (18.40–99999)]; however, the distribution within *J. jaculus* was characterized by multiple peaks (Fig. 4). The goodness of fit test for deviation from the expectation under the Sudden Expansion Model rejected only the model for the data set including both cryptic species ($P = 0.022$; $P > 0.5$ for separate analyses for *J. deserti* and *J. jaculus*). The expansion of *J. deserti* in to the North-West Sahara was estimated to have occurred between 19 000 and 45 000 years ago. The multinomial distribution of *J. jaculus* *cyt b* haplotypes suggests multiple colonizations and further structuring of the population. Also, two haplotype groups within *J. jaculus* (but one within *J. deserti*) were detected with the reconstructed gene networks (Fig. 5), which corresponded to two picks observed on the mismatch distribution (Fig. 4) and multiple clades on the phylogenetic trees (Fig. 2; see also Supporting information, Fig. S1). The Tajima's *D* and Fu's F_S statistics were negative within cryptic species analyses and, in most cases, significantly deviated from zero (Table 1). Unimodality of the mismatch distribution of the number of pairwise differences between *cyt b* haplotypes, as observed within *J. deserti*, and negative values of tests of selective neutrality are expected for populations undergoing recent growth or that are exposed to severe selective pressures (Excoffier, Foll & Petit, 2009).

There were significant difference in the variance of occurrences of *J. deserti* and *J. jaculus* for the annual mean temperature ($t = 2.309$, $P = 0.013$, d.f. = 51), whereas no significant differences were found for annual precipitation ($t = 0.508$, $P < 0.307$, d.f. = 38) or land-cover categories ($\chi^2 = 4.993$, $P = 0.288$, d.f. = 4). Specimens of *J. deserti* were more frequently found in cooler areas than *J. jaculus* (Fig. 6).

DISCUSSION

The results obtained in the present study confirmed the co-existence of two lineages or cryptic species described within lesser Egyptian jerboas in North-West Africa (Fig. 1). The split was evident based on mitochondrial and nuclear markers, although the lineages showed little differences in preference to environmental conditions. The genetic polymorphism and reconstructed demographic histories showed marked differences in the age of expansion between species and suggested long distance migrations as the likely mechanisms shaping genetic variation within the African jerboas.

According to taxonomic norms, the two described lineages could be named *J. jaculus* (Linnaeus, 1758) and *J. deserti* Loche, 1867, which has been suggested in the literature (Ranck, 1968; Shenbrot *et al*., 2008). The current phylogenetic reconstructions of unlinked genes confirmed the existence of two species, previously recognized as the lesser Egyptian jerboa, *J. jaculus* (Linnaeus, 1758). The cryptic species formed monophyletic clades, concordant between nuclear and mitochondrial genes, and statistically supported in three different phylogenetic methods (Figs 2, 3). The between species divergence of *cyt b* was high (10.6%), and above the divergence level usually observed between species (mean 9.55% of *cyt b* sequence divergence between sister rodent species), which generally meets the expectation of the Genetic

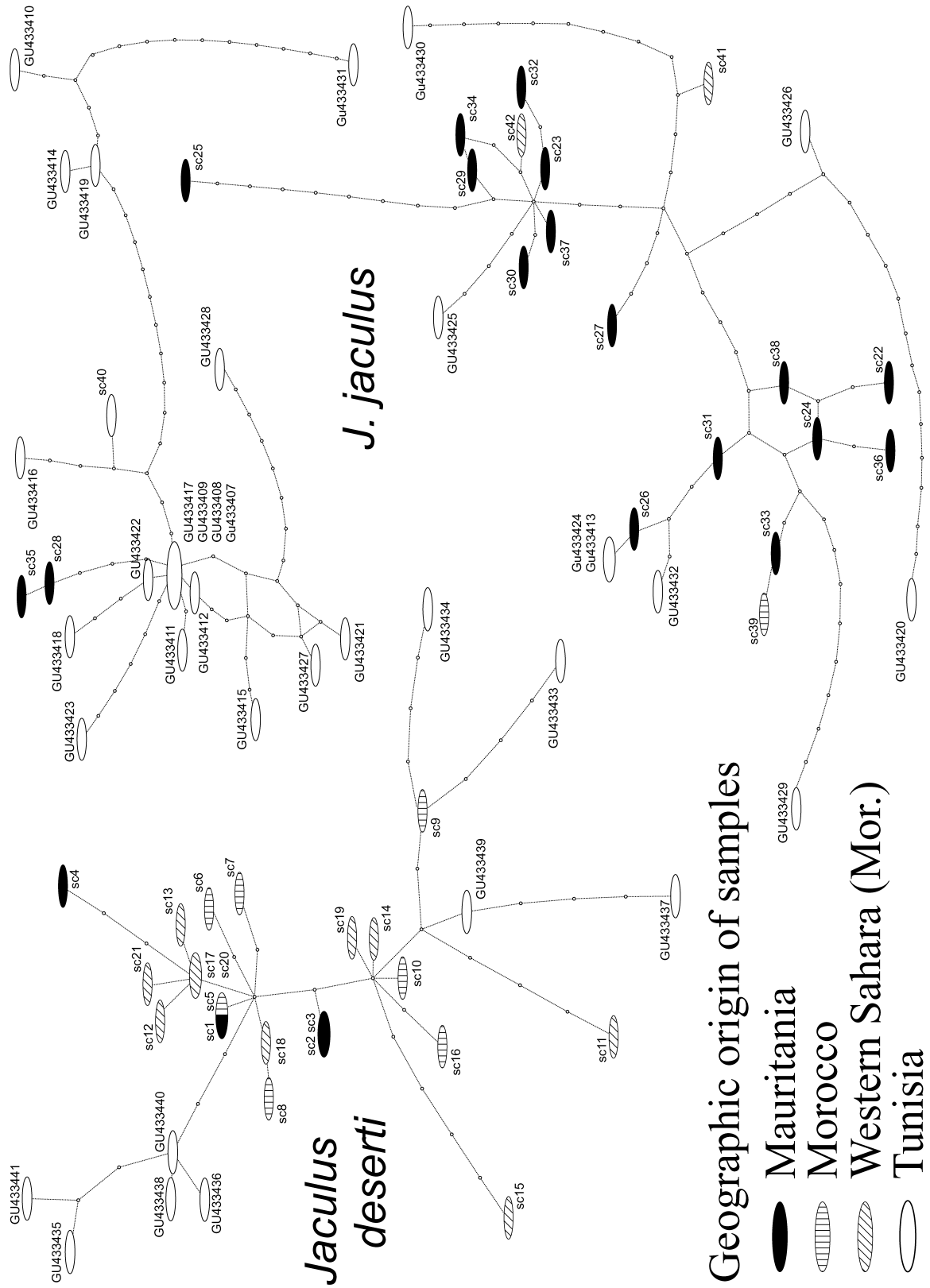

Figure 5. Haplotype networks for cytochrome *b* (*cyt b*) sequences of *Jaculus jaculus* and *Jaculus deserti*. Each oval represent one haplotype and its size is proportional to the number of samples. Numbers near ovals represents sequenced samples (Fig. 1; see also Supporting information, Table S1) or GeneBank accession numbers (see Supporting information, Fig. S1). Small circles on the branches indicate hypothetical haplotypes.

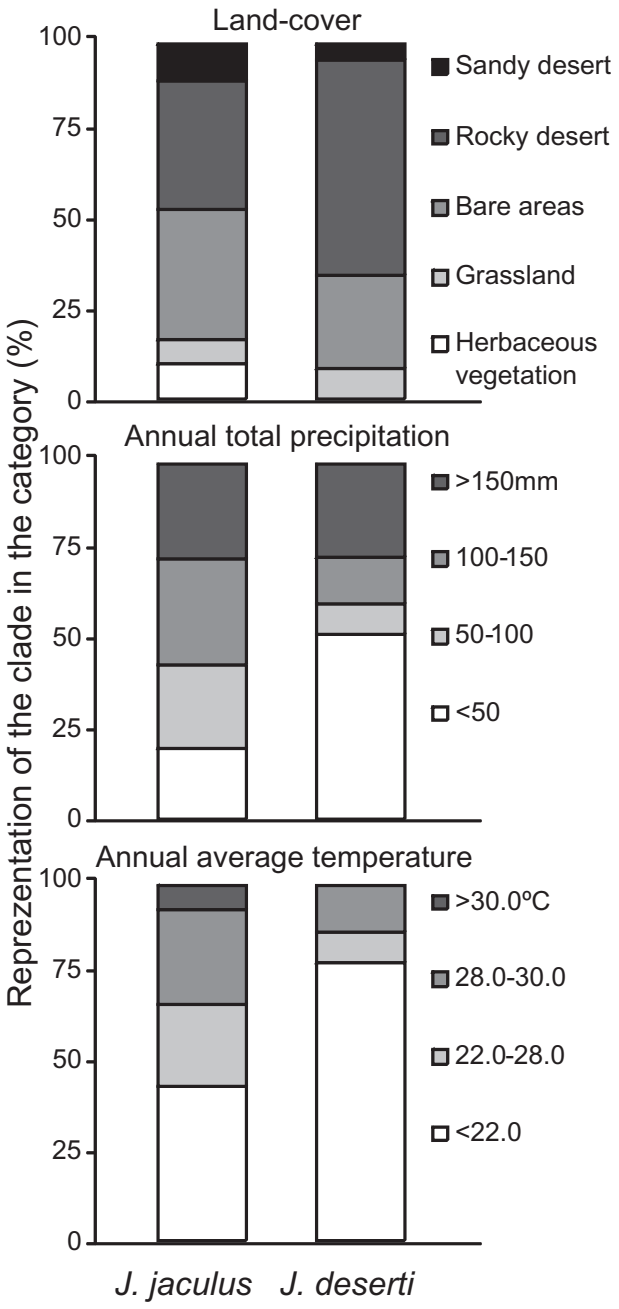

Figure 6. Relative number of observations of *Jaculus jaculus* and *Jaculus deserti* in each category of three environmental factors: land-cover, annual total precipitation, and annual mean temperature.

Species Concept developed for mammals (Bradley & Baker, 2001; Baker & Bradley, 2006). The divergence in *vWF* sequences was smaller (1.3%) than that for the *cyt b* gene, as expected for this slowly evolving nuclear marker (Huchon, Catzeflis & Douzery, 1999). However, the observed divergence in *vWF* was higher than that observed between other rodent species. The divergence between *Microtus agrestis* and *Microtus socialis*, relatively distantly-related species (Jaarola *et al*., 2004), was only 1.1% (difference calculated from GenBank sequences: FM162067, FM200055). Accordingly, the differences between two other rodents species, also quite distantly-related (*A. hermonensis* and *ponticus*: AB303283, AB303282; Michaux *et al*., 2002) was much lower (0.8%) than the variation detected between *J. deserti* and *J. jaculus*. Given these values that the divergence between distantly-related *Jaculus* and *Dipus* rodents was only 6.5%, it is likely that the difference of 1.3% refers to the distinction of closely-related species.

The high variation in *cyt b* allowed detailed analyses of haplotype polymorphism. In general, the result of mismatch distribution among haplotypes was congruent with phylogenetic analyses showing the existence of two main mitochondrial haplogroups, referring to two cryptic species (Fig. 4). The sequence variation among *J. deserti* haplotypes shows a pattern not congruent with a model of population equilibrium (i.e. bell-shaped mismatch distribution and negative Tajima's D and Fu's F_S values; Fig. 4, Table 1), suggesting a relatively recent expansion and colonization event [$\tau = 4.4$ (95% confidence interval = 2.7–5.8)]. Assuming one generation per year and substitution rates characteristic for mammals (Nabholz *et al*., 2008), the age of the expansion of *J. deserti* in to the North-West Sahara occurred between 19 000 and 45 000 years ago. This time interval coincides with the drastic environmental change of the Sahara; a wet period began approximately 40 000 years ago and ended approximately 19 000 years ago, when the hyper-arid period began (Le Houérou, 1997). Climate changes of such magnitude are known to play crucial roles in shaping species distributions and could have triggered the population expansion of *J. deserti*. At least two clear peaks in the pairwise differences between haplotypes within *J. jaculus* suggest multiple colonizations and structuring within the lineage. Mismatch analysis [$\tau = 10.5$ (95% confidence interval = 5.0–13.0)] of the main *J. jaculus* lineage suggested that the expansion to its current distribution predated that of *J. deserti*, occurring sometime between 42 000 and 98 000 years ago, which also may have been triggered by climate change in the Sahara (Dumont, 1982; Le Houérou, 1997). The reconstructed networks showed a lack of clear geographical structuring of lineages and cryptic species (Fig. 5), and suggested relatively recent long distance migrations between distant locations, such as between central-western Mauritania and Tunisia (e.g. samples 28 and 35 in Figs 1, 5).

The results of the present study clearly show that the history of the two cryptic species is substantially different: *Jaculus deserti* is relatively

homogeneous and has undergone recent expansion, whereas multiple lineages were detected within *J. jaculus*. The age of divergence between *J. deserti* and *J. jaculus* (between 1.8 and 4.1 Mya) is very imprecise because no attempts have been made to estimate the substitution rate in these rodents and the fossil records necessary for calibrating phylogenetic trees are unavailable. Nevertheless, it is likely that the species originated near the Upper Pliocene-Lower Pleistocene boundary. The first evidence of aridity in North Africa is known from the Mid-Upper Pliocene in the southern Sahara from aeolian deposits and fossils of xerophytic vegetation (Le Houérou, 1997). The *Jaculus* species may have originated around that time and diversified during subsequent periods of aridification occurring in North Africa. The climate fluctuations in North Africa likely have generated vicariance (separation) events between initial populations of jerboas, even though species currently occur in sympatry in many localities. Parapatric speciation is also suggested by small differences in environmental preferences between *J. jaculus* and *J. deserti* (Fig. 6), showing that ecological niche displacement may not yet have evolved completely. From environmental analyses, it appears that *J. jaculus* can populate wider range of habitats then *J. deserti*; however, only differences in annual mean temperature between sample locations were significant. It is therefore plausible that *J. deserti* may have evolved in an isolated and small population, and underwent population growth and expansion relatively recently. On the other hand, the genetic polymorphism within *J. jaculus* suggests that demographic and expansion–contraction processes may have influenced this species in more complicated ways than *J. deserti* (Fig. 4). Particularly, at least two divergent lineages are present within *J. jaculus*, although their geographical structuring is not very clear (Fig. 5), allowing the hypothesis that migration between distant geographical localities may be common.

Conclusions

The results of the present study support the hypothesis that North-West Africa is populated by two cryptic species recognized within the lesser Egyptian jerboa. The deep genetic distance evident from the mitochondrial markers supports the coexistence of two species. Divergence in nuclear sequences was less, although it also occurred on the species level. Reconstructed genealogies suggested no hybridization between species, and weak ecological differences imply a relatively recent divergence. We showed that genetic polymorphism is hierarchically structured within *Jaculus* species. This pattern is probably related to the times of colonizations by different lineages and generated by environmental and climatic shifts that have frequently occurred in North Africa since the Pliocene. Sympatry of distant lineages suggests that, after the initial diversification and perhaps speciation, lineages underwent population growth and colonized wider areas. Identification of the main geological and climatic events that may have affected (and may continue to affect) species distributions and diversification will help in understanding the ecological and evolutionary forces driving genetic polymorphism and speciation within the African jerboas and other desert species in North-West Africa.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Consensus phylogram reconstructed from the partial sequences of the cytochrome *b* gene, including original and GenBank sequences.
Figure S2. Consensus phylogram reconstructed from concatenated sequences of cytochrome *b* and von Willebrand factor.
Table S1. Samples included in the present study and their GenBank accession numbers.

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